

## Potential of *Beauveria bassiana* Lowland Isolates against *Spodoptera litura* in Tobacco Plant

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**Abstract.** The study of the ability of *Beauveria bassiana* lowland isolates as biological control of *Spodoptera litura* larvae needs to be done because of the specific characteristics of biological control that are site specific and host specific. The research objectives are: 1) analyze *B. bassiana* lowland isolates which were effective in suppressing *S. litura* attacks in the field and 2) analyze the ability of *B. bassiana* lowland isolates to increase the production of Kasturi tobacco. The study was conducted using Randomized Block Design (RAK) with 4 replications. Treatment consisted of application: (1) *B. bassiana* lowland isolate strain 715 0.4 gram/liter, (2) *B. bassiana* lowland isolate strain 725 0.4 gram/liter, (3) *B. bassiana* lowland isolate strain Jember spore density  $10^9$  spores/ml, (4) *B. bassiana* lowland isolate strain Jombang spore density  $10^9$  spores/ml, (5) *B. bassiana* highland isolate strain Temanggung spore density  $10^9$  spores/ml and (6) synthetic chemical insecticides 2 gram/liter. The result showed that *B. bassiana* lowland isolate strain Jombang succeeded in suppressing leaf damage due to *S. litura* attack 50% - 60% compared to other *B. bassiana* isolates and has the potential to be developed into biological control products against *S. litura* in lowland Kasturi tobacco plants.

### 1. Introduction

*Spodoptera litura* larvae can reduce the productivity of Kasturi tobacco plants by eating leaves marked by holes in the affected part of the plant. Entomopathogen fungi *Beauveria bassiana* had the high capability as biological controllers of tobacco leaf-eating larvae and is known to have so many hosts but still has specific host limitations and specific locations. Some research has shown that the application of *B. bassiana* had the high mortality of *S. litura* larvae between 50% - 90% [1], [2], [3].

The ability of *B. bassiana* to control pests from the order of Lepidoptera such as *S. litura* can still be increased through genetic activity [4]. The pathogenesis of entomopathogen fungi is supported by enzyme during the invasion period [5]. The hyphae of *B. bassiana* are able to penetrate into the host cuticle due to secreting the extracellular type of enzymes like lipase, protease and chitinase [6]. The study about *B. bassiana* pathogenicity has been widely investigated but studies on the ability of *B. bassiana* lowland isolates are still very limited. Therefore, the study to find *B. bassiana* lowland isolates that are most effective against *S. litura* larvae in Kasturi tobacco plants needs to be done to be able to increase the production of tobacco plants while still taking into account the quality of the environment.



The research objectives are (1) analyze *B. bassiana* lowland isolates which were effective in suppressing *S. litura* attacks in the field and (2) analyze the ability of *B. bassiana* lowland isolates to increase the production of Kasturi tobacco.

## 2. Material and Methods

The study has been carried out at the Plant Protection laboratory and tobacco field at the Jember State Polytechnic (*S 809'34,884 "E 113043'24.7944"*) from May to November 2018. The research used a single factor of Randomized Block Design and 4 replications. Treatments: (1) *B. bassiana* lowland isolate strain 715 0.4 gram/liter, (2) *B. bassiana* lowland isolate strain 725 0.4 gram/liter, (3) *B. bassiana* lowland isolate strain Jember spore density  $10^9$  spores/ml, (4) *B. bassiana* lowland isolate strain Jombang spore density  $10^9$  spores/ml, (5) *B. bassiana* highland isolate strain Temanggung spore density  $10^9$  spores/ml and (6) synthetic chemical insecticides 2 gram/liter.

### 2.1. Preparation of *B. bassiana*

*B. bassiana* isolates were collected from several lowlands: (1) strain 715 was isolated from Coleoptera and strain 725 was isolated from Lepidoptera in Kaliwining Jember with altitude 45 meters above sea level (the product form of freeze-dried spores) [7], (2) strain Jember was isolated from Coleoptera in Summersari Jember with altitude 89 meters above sea level (the product form of corn rice multiplication formulation), (3) strain Jombang was isolated from Lepidoptera in Mojoagung Jombang with altitude 44 meters above sea level (the product form of corn rice multiplication formulation) and (4) strain Temanggung was isolated from Coleoptera with altitude 675 meters above sea level (the product form of corn rice multiplication formulation).

### 2.2. Preparation of Kasturi Tobacco Plant

Preparation and implementation procedures in concert with the method of Tobacco Good Practice (GTP). Prepare the land for tobacco plants Kasturi to choose a flat land, open and a height of 89 meters above sea level. The Kasturi tobacco seed which is a type of chopped tobacco that is widely planted in the Jember area is planted and transplanted at the age of 45 days in a polybag containing planting media (topsoil: compost: sand) which is suitable for the growth. Placement of tobacco polybags with a single spacing (100 cm x 45 cm) and in line with the research layout. Maintenance tobacco plants are conducted in pursuance of Standard Operating Procedures for technical procedures include covering tobacco maintenance, watering, fertilizing, cultivating and controlling plant pests and diseases [8].

### 2.3. Application of *Beauveria bassiana*

The application procedure to prepare the *B. bassiana* from each strain lowlands and highlands follow with a predetermined concentration then mixing the solution with an adhesive compound until smooth and homogeneous. Applications treatments by spraying a solution in line with the determination of the tobacco crop research plots each week. Applications entomopathogen fungus carried out in the afternoon to avoid the effect of sunlight on *B. bassiana* and chemical application is done in accordance with standard operating procedures of the manufacturer.

### 2.4. Observation

The parameters observed in this study are (1) the intensity of pest attack on new leaves is open, which is calculating the percentage of damage to the leaves due to pest attack before and after the control application. The formula for calculating damage intensity:

$$P = \frac{\sum (n \times v)}{N \times Z} \times 100\%$$

P = leaf damage intensity (%)

n = number of leaves that have the same damage category

v = score value/category based on the area of the affected leaves

N = number of leaves observed per plant

Z = the highest damage score / category value

Value scale / damage scoring [9]:

0: no pest attack

1: damage area of leaves more than 0- 25%

3: leaf damage area of more than 25 - 50%

5: leaf damage area is more than 50 - 75%

7: leaf damage area is more than 75 - 100%

(2) Growth of tobacco plants on plant height and (3) Leaf growth include the number of leaves, leaf length, leaf width.

### 3. Result and Discussion

#### 3.1. Leaf Damage Intensity

*S. litura* larvae were found since tobacco plants aged 9 days after planting and the population fluctuated during the vegetative phase of the plant which then decreased after tobacco plants entered the generative phase. The decline in population is thought to be due to the influence of treatment applications also related to the plant growth phase because *S. litura* attacks the leaves of plants that develop a lot in the vegetative phase of the plant. The direct impact of attacks is seen on the leaves. Pests attack by eating leaves that are marked by holes in the affected part of the plant. In severe attacks will cause harvest failure.

**Table 1.** The average intensity of Kasturi tobacco leaf damage due to *S. litura* attack

Treatment	14 dap	28 dap	42 dap
P1 (lowland isolate strain 715)	2.72 a	6.22 b	6.58 b
P2 (lowland isolate strain 725)	0.70 a	2.89 a	5.33 b
P3 (lowland isolate strain Jember)	6.93 b	7.48 b	4.61 ab
P4 (lowland isolate strain Jombang)	0.00 a	4.74 ab	2.43 a
P5 (highland isolate strain Temanggung)	0.93 a	6.31 b	4.73 ab
P6 (chemical insecticide)	1.10 a	1.05 a	1.64 a

The number accompanied by the same alphabet in the same track shows no significant difference based on the LSD (5%)

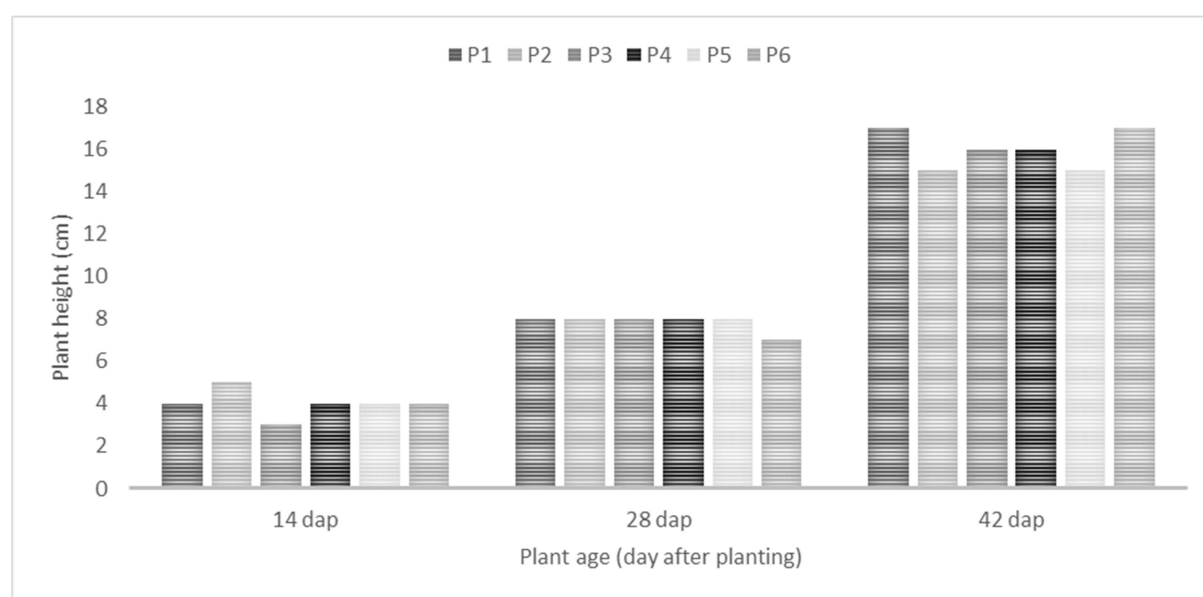
The level of damage Kasturi tobacco leaves aged 14 days after planting as a result of the attack of *S. litura* showed that the application *B. bassiana* lowland isolate strain Jombang was able to suppress the attack rate to 0% which is not significant with other treatments, except the highest level of leaf damage on the application *B. bassiana* lowland isolate strain Jember reach 6.9%. The level of damage increases with age leaf plants because the leaves are elongated growth and widening seen on plants 28

days and 42 days after planting (Table 1). The average damage of Kasturi tobacco leaves at 14, 28 and 42 days after planting respectively 2.06%, 4.78% and 4.22%. There was an increase in average leaf damage at 28 days after planting but the average leaf damage decreased at 42 days after planting. The application of *B. bassiana* lowland isolate strain Jombang succeeded in suppressing leaf damage due to the attack of *S. litura* reach 50% - 60% compared to the application of other *B. bassiana* isolates. The treatment of insecticides is not significantly different from the treatment of *B. bassiana* lowland isolate strain Jombang.

Chemical insecticide active ingredient used lambda-cyhalothrin 106 grams/liter and thiamethoxam 141 grams/liter with transmlar working mechanism into the network at the same plant are toxic contact and stomach poison to the target pest. Quickly absorbs into the plant tissue that is not easily washed away by rainwater [10]. Treatment *B. bassiana* lowland isolates strain Jombang was isolated from the order Lepidoptera pests at an altitude of 44 meters above sea level shows a lower level of leaf damage. This shows that the power of entomopathogen fungi in suppressing the level of pest attack is influenced by the high similarity of the place and host suitability. This experiment arranged at altitude 89 meters sea level including lowland area. One characteristic of *B. bassiana* as a biological controller is the specific nature of the host and the specific locations that have limited because only able to infect insects suitable host [11]. Entomopathogenic fungi have bio-trophic linkages with insect host and can act as a saprophyte [12]. Conidia entomopathogen fungi that enter the body of the host insect through feeding larvae will be digested and the infection sometimes does not occur before excretion due to incompatibility of intestinal specific virulence factors [4]. The fungus infects the host insect through the cuticle penetration into the host by way of adhesion [11]. Pathogenicity of entomopathogen fungi depend on the activity of lipase, protease and chitinase which will degrade the integument of insects [12]. Virulence ability is strongly influenced by extracellular enzyme activity. Chitinase is an enzyme that has the most role in decomposing the chitin layer from insect cuticles [6].

### 3.2. Plant Height

Plant height is one measure of plant growth is affected by photosynthesis rate. The rate of photosynthesis can be approached by counting the number of leaves. The process of photosynthesis will produce primary metabolites used for plant metabolism resulting from the growth and the development of plants. Ability to process light energy leaves accepted into carbohydrates by photosynthesis at temperatures referents and its value is affected by plant genetic and environmental conditions [13].



**Figure 1.** *B. bassiana* lowland application against the Kasturi tobacco plant height

Application of *B. bassiana* all isolates do not affect the height of tobacco plants as shown in Figure 1. This outcome indicates that the biological control application is still able to maintain plant growth. Biological controllers with *B. bassiana* will increase the natural stability of the ecosystem and support the existence of other natural enemies on the land and research plots. Several types of natural enemies other than biological control were applied to research plots. The existence of natural enemies in the ecosystem will support natural control running in balance and pest populations can be maintained at a non-detrimental boundary.

The application of biological control in an ecosystem will increase biodiversity in the ecosystem. Biodiversity causes a dynamic interaction between supporting components so that a balance condition is formed. In dynamic equilibrium conditions, there is no type of organism that becomes dominant because each type of organism is naturally controlled by other organisms and other natural control components [14]. Utilization entomopathogen fungus is one of alternative pest control. Biological control applications of fungi and selective insecticides will reduce dependence on chemical insecticides and reduce residual effects on agriculture [15].

### 3.3. Leaf Growth

Tobacco production is in the form of leaves that are widely used for industry. Tobacco Kasturi has low nicotine content of 1-3% in the leaves. Tobacco plant growth can be assessed through leaf growth, including the number, length and width of leaves.

**Table 2.** *B. bassiana* application for the average growth of the number, width and length of Kasturi tobacco leaves

Treatment	Leaf width (cm)			Leaf number (leaf blade)			Leaf length (cm)		
	14 dap	28 dap	42 dap	14 dap	28 dap	42 dap	14 dap	28 dap	42 dap
P1 (lowland isolate strain 715)	5.18	9.43 bc	13.03	3.71	5.00	8.83	9.49	16.95	23.96
P2 (lowland isolate strain 725)	5.44	10.7 abc	12.32	4.54	5.21	8.13	10.04	18.70	22.38
P3 (lowland isolate strain Jember)	5.22	9.54 bc	11.22	3.83	4.75	8.63	9.99	17.17	22.73
P4 (lowland isolate strain Jombang)	5.36	10.71 ab	12.44	4.17	5.38	8.63	10.11	18.66	24.00
P5 (highland isolate strain Temanggung)	4.79	8.71 c	12.32	3.96	4.83	8.63	9.16	15.67	22.86
P6 (chemical insecticide)	5.51	11.64 a	13.54	4.33	5.04	8.46	10.26	20.00	24.59

The number accompanied by the same alphabet in the same track shows no significant difference based on the LSD (5%)

Leaf number and leaf length at 14, 28 and 42 days after planting is not affected by the treatment of *B. bassiana*. It shows that the application of entomopathogen fungi does not affect the growth and development of Kasturi tobacco plants. Meanwhile, application *B. bassiana* affect leaf width at 28 days after planting. Application of chemical insecticide, *B. bassiana* lowland isolate strain Jombang and *B. bassiana* lowland strain 725 shown has a wider leaf width compared to other treatments.

Based on Table 2 shows the treatment only affects the width of the leaf at 28 days after planting. Treatment applications to give effect to the width of the leaves for larvae of *S. litura* attack by eating the leaves of tobacco plants. Perforated tobacco leaves due to the feeding activity of *S. litura* larvae. This will increase the risk of decreasing the production of tobacco plants considering the results of tobacco plants are leaves that become raw materials for cigarettes, drugs or cosmetics. *S. litura* known as the armyworm because in one egg groups contain about 350 eggs and egg reaches 2000-3000 the number of all the grains. The eggs will hatch after 3-5 days [16].

The width of the large leaves contained in the application showed that the two strains of *B. bassiana* lowland isolate strain Jombang and *B. bassiana* lowland isolate strain 725 minimize damage to tobacco leaves so that it has the potential to be developed as a biological control for *S. litura* larvae in Kasturi tobacco plants in the lowlands. The virulence ability of *B. bassiana* to resist the immune response of host insects is supported by several kinds of proteins and enzymes through molecular and

physiological mechanisms [6]. Host insect integument is degraded through the ability of enzymes activity such as lipase, protease and chitinase [12].

Leaf number, leaf width and leaf length at 42 days after planting are not affected by the application. It shows the treatment was able to maintain the growth and development of leaf tobacco plant including *B. bassiana* highland isolate strain Temanggung as one strain originating from the highlands. The ability of *B. bassiana* highland isolates strain Temanggung to keep the average growth of the number, width, and length of tobacco leaves proved that *B. bassiana* have the power to adapt to the altitude. *B. bassiana* can adapt to different environments with the adjustment and activation of sets of genes that have been defined correctly [17]. The virulence of *B. bassiana* against *S. litura* larvae can be significantly improved through genetic transformation. Conidia result of the genetic transformation is spread widely by the vector so it can function as a controller *S. litura* [4].

Conidia viability of entomopathogen fungi can be maintained when formulated in the form of pure conidia for more easily applied and has a higher accuracy compared to other traditional methods [18]. Entomopathogen fungi more effectively applied to the pests that have gregarious eating patterns, such as *S. litura* as it will accelerate the spread of infection. It can be applied in the integrated pest management program [15].

#### 4. Conclusion

*B. bassiana* lowland isolate strain Jombang succeeded in suppressing leaf damage due to *S. litura* attack 50% - 60% compared to other *B. bassiana* isolates and has the potential to be developed into biological control products against *S. litura* in lowland Kasturi tobacco plants.

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